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Effect of 2% chlorhexidine gluconate cavity disinfectant on microtensile bond strength of tooth-coloured restorative materials to sound and caries-affected dentin

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Short title: *Effect of chlorhexidine on adhesion of restorative materials to dentin*

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Abstract: This study evaluated the effect of 2% chlorhexidine gluconate based cavity disinfectant (CHX) on the microtensile bond strength (μTBS) of glass ionomer, resin-modified glass ionomer and packable resin composite to sound and caries-affected dentin. Sound and occlusal caries-affected human third molars (N=36, n=3 per group) were randomly divided into three experimental groups to receive one of the following restorative materials; **a)** Glass ionomer (Ketac Molar, 3M ESPE; GI), **b)** Resin-modified glass ionomer (Vitremer, 3M ESPE; RMGI) and **c)** Packable resin composite (Surefil, Dentsply; PRC) with a bonding agent (Prime Bond NT, Dentsply De Trey). Caries was removed using a caries-detecting dye (Caries Detector, Kuraray Medical Ltd.) and flat dentin surfaces were achieved by finishing up to 1200-grit silicon carbide abrasive. Half of the teeth in each group received 2% CHX (Consepsis, Ultradent). Dentin surfaces were built-up with the respective materials incrementally and were sectioned with a slow-speed saw into multiple beams. The beams were subjected to microtensile bond strength test (μTBS) (0.5 mm/min) in a Universal Testing Machine. The data were analyzed using 2-way ANOVA and Tukey`s tests. For each restorative material, μTBS results were not affected by the application of CHX ($P>0.05$) on both sound and caries-affected dentin ($P>0.05$). PRC in combination with the corresponding bonding agent showed significantly higher results ($P<0.05$) than those of GI and RMGI, on sound and caries-affected teeth, respectively. Cohesive failure in dentin was not observed in any of the groups. The use of 2% chlorhexidine-based cavity disinfectant did not impair the adhesion of the restorative materials tested to either sound or caries-affected dentin.

Keywords: Cavity disinfectant, chlorhexidine, composite resin, glass ionomer cement, microtensile bond strength

Introduction

Many materials are available for the restoration or repair of missing dental hard tissues due to caries. Yet, microbial growth under restorations is still considered as a biological problem in dentistry. Various techniques have been suggested to reduce or remove microorganisms underneath the restorations. The application of chlorhexidine gluconate containing disinfectants (CHX) following cavity preparation is one of the commonly suggested methods to eliminate residual bacteria [1,2]. CHX, a biguanide antimicrobial agent, has been reported to inhibit the activities of MMP-2, MMP-8 and MMP-9 [3]. For etch-and-rinse adhesives, CHX may be applied to the demineralized dentin directly or incorporated into an acidic conditioner prior to the application of adhesives, which has been shown to be effective for reducing degradation of resin-dentin bonds after aging [4,5,7]. However, the reports are controversial whether such an antibacterial agent may affect the adhesion of the restorative material to dentin or not [2,4,5,8].

Matrix metalloproteinases (MMP), one of the host derived collagen-degrading proteases, is suspected to be involved in the degradation of unprotected collagen within incompletely resin-infiltrated acid-etched dentin [9,10], which could explain the progressive degradation of the hybrid layers seen in numerous in vivo [5,11,12] and laboratory studies [10,13]. In addition, in the absence of bacteria, persistent collagenolytic activity exhibited by unbonded, partially demineralized human dentin substrates was associated with a morphological disintegration of dentinal collagen fibrils. CHX, a potential MMP inhibitor, was shown to reduce the dentinal collagenolytic activity minimizing the auto-degradation of the exposed collagen fibrils within incompletely-formed hybrid layers [5,11] thereby, contributing to the long-term stability of the hybrid layer and bond strength.

The objectives of this study were to a) evaluate the effect of 2% CHX on the microtensile bond strength of glass ionomer, resin-modified glass ionomer and packable resin composite with its corresponding bonding agent to sound and caries-affected dentin and b) analyze the failure types after debonding. The null hypothesis tested was that CHX application would not impair adhesion of the tested restorative materials on sound and caries-affected dentin.

Materials and Methods

Sound and occlusal caries-affected human third molars (N=36, n=3 per group) were obtained. After radiographic evaluation, only the carious teeth were selected where the dentin caries extend no further than the middle one-third of the dentin thickness. The carious lesion was removed by means of a slow-speed diamond disc (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water to achieve flat dentin surface. Caries detecting dye (Caries Detector, Kuraray Medical Ltd., Tokyo, Japan) was used to control whether all carious dentin had been removed. In order to obtain carious affected dentin, grinding was performed in such a way that visual examination was combined with staining using caries detector dye. Subsequently, the caries affected dentin was distinguished using visual screening (slightly pink coulored areas following grinding) and probing (hard surface contact on probing) [14,15]. It was ensured that caries affected dentin was hard to an explorer and stained bright red caries detector dye was no longer visible. Caries affected dentin was not re-checked with hardness measurements.

For the sound teeth, also initially radiographs were made to evaluate the level of dentin to be used for bonding, and made sure that up to approximately the middle one-third of the dentin was intact. Dentin surfaces were prepared as described for carious dentin.

Bonding procedures

Twelve specimens were prepared for each group by the same clinician (6 caries affected and 6 sound dentin) [16]. They were then randomly divided into two subgroups according to the application of disinfectant. After washing the exposed flat occlusal dentin surfaces and drying with oil-free compressed air, the restorative materials namely, a) Glass ionomer (Ketac Molar, 3M ESPE; GI), b) Resin-modified glass ionomer (Vitremer, 3M ESPE; RMGI) and c) Packable resin composite (Surefil, Dentsply; PRC) were applied in control groups. The materials used in this study are presented in Table 1. Experimental design of the study consisting the groups and the treatment methods are presented in Fig. 1.

Application procedures were as follows:

Ketac Molar (GI): Dentin surfaces were conditioned with Ketac Conditioner for 30 s, rinsed for 10 s and air-dried for 5 s. Ketac Molar (3M ESPE, Seefeld, Germany) powder and liquid were hand-mixed according to the manufacturer's instructions and applied to a thickness of 5 mm and width of 6 mm on the dentin surfaces. Ketac glaze was then applied, photo-polymerized for 10 seconds with a light emitting diode (LED) (Elipar, Trilight, 3M ESPE, Dental Products, St. Paul, USA).

Vitremer (RMGI): Vitremer Primer was applied to dentin surfaces for 30 s, air-dried for 15 s and photo-polymerized for 20 s. A 5 mm thick and 6 mm width build-up was created incrementally with Vitremer and photo-polymerized for 40 s.

Surefil (PRC): Etchant (36% ortophosphoric acid) was applied to the dentin surfaces for 15 s, and rinsed for 15 s. One layer of bonding agent (Prime Bond NT, Dentsply De Trey, Konstanz, Germany) was applied for 20 s, air-dried for 5 s, and photo-polymerized for 20 s. Surefil (Dentsply De Trey, Konstanz, Germany) was placed incrementally on dentin resin build-ups were created to a thickness of 5 mm and width of 6 mm. Each incremental layer was photo-polymerized for 40 s with the same polymerization device as the other groups.

In the disinfected groups, 2% chlorhexidine gluconate containing disinfectant (Consepsis Ultradent, South Jordan, UT, USA) was used as cavity cleaner and applied with its applicator for 60 s, according to the manufacturer's instructions. Then, the dentin surface was only dried gently.

Initially, dentin was conditioned in GI and PRC groups and Vitremer primer was applied in the RMGI group.

The specimens were stored in distilled water at 37°C for 24 h.

Microtensile bond strength (μTBS)

Each tooth was sectioned with a slow-speed diamond disc under water-cooling into multiple beams (approximately 1 mm^2), with the 'non-trimming' version of the microtensile test. The roots of the teeth were not removed for better retention during obtaining beams. When the external beams from the periphery were excluded, 20-30 beams were expected to obtain from the central part of each tooth-composite assembly. Beams achieved from peripheral dentin were not included to the study. Also, pre-test failures were not involved in the statistics.

Beams were fixed to the jig (Bencor Multi-T, Danville Engineering Co., Danville, CA, USA) of the Universal Testing Machine (Model 5544, Instron Corp., Canton, MA, USA) using cyanoacrylate glue (Model Repair II Pink, Dentsply-Sankin, Ohtawara, Japan) and tensile force was applied at a cross-head speed of 0.5 mm/min until failure. The cross-sectional area at the site of failure was measured to the nearest 0.01 mm with a digital caliper (Model CD-6BS, Mitutoyo, Tokyo, Japan), from which the microtensile bond strength was calculated and expressed in MPa.

Failure modes were evaluated under the stereoscopic microscope (LG-PS2, Olympus Co., Tokyo, Japan) at at x40 magnification and classified as follows: Score 1= adhesive failure between the restorative material and dentin; Score 2= partially adhesive failure between dentin and restorative material and dentin accompanied with cohesive failure in the restorative material (mixed); Score 3= cohesive failure of the restorative material only; Score 4= cohesive failure in dentin.

The most representative specimens from each group were prepared and observed under Scanning Electron Microscope (SEM) (JSM-5500; Jeol Ltd., Tokyo, Japan).

Statistical analysis

Descriptive statistics were computed and test of normality was performed using Kolmogorov-Smirnov and Shapiro- Wilk's tests. The means of each group were analyzed by 2-way analysis of variance (ANOVA), with microtensile bond strength as the dependent variable and restorative material types (3 levels: GI, RMGI, Prime and Bond NT-PRC) and CHX application (2 levels: sound and carious dentin) as the independent factors (Statistix 8.0 for Windows, Analytical Software Inc, Tallahassee, FL, USA). Multiple comparisons were made by Tukey`s adjustment test. The tooth was used as the unit of analysis and not number of microtensile test sticks. *P* values less than 0.05 were considered to be statistically significant in all tests.

Results

Data were normally distributed in all groups. Pre-test failures were more common in GI followed by RMGI and Prime and Bond NT-PRC. μTBS results were not affected by the application of CHX within each restorative material ($p>0.05$) for sound and caries-affected dentin compared to the groups where no CHX was applied prior to bonding restorative materials ($p>0.05$) (Table 2). Without CHX application, PRC (39.6±5.3, 38.7±5.4) showed significantly higher results ($p<0.05$) than those of GI (12.2±6.3, 11.1±7.2) and RMGI (19.8±6.5, 18.3±6.4), on sound and caries-affected teeth, respectively. GI and RMGI did not show significant differences in all conditions ($p>0.05$).

Cohesive failure in dentin (Score 4) was not observed in any of the groups (Table 3). While adhesive failure between the restorative material and dentin (Score 1) was more frequently observed in PRC than those of GI and RMGI, cohesive failure of the restorative material only (Score 3) was more common in GI followed by RMGI.

Discussion

The results of this study indicated that application of CHX disinfectant did not decrease the μTBS of the tested materials to both sound and caries-affected dentin substrates compared to the control group. Thus, the null hypothesis was accepted.

The use of CHX containing products as a cavity disinfectant has gained popularity; however, studies have reported that adhesion of the restorative materials could be impaired by the application of disinfectants [17]. Results of laboratory studies found in the literature is controversial regarding whether or not to use this agent and there is not much information on how these agents may affect the bond of glass ionomer materials. In the present study, the influence of a CHX-based cavity disinfectant was investigated on the μTBS of three different tooth-colored restorative materials to sound or caries-affected permanent dentin. The results indicated that the disinfectant did not interfere with the μTBS of the tested materials to both dentin substrates. When shear bond test was used, other studies also showed that the application of CHX did not have a negative effect on the bond strength of adhesive systems [1,18]. On the other hand, one study even reported increased shear bond strength when CHX was used [19]. De Castro et al. [18], reported that 2% CHX solution, applied before or after acid etching of the dentin, did not interfere with the μTBS of composite resin to the dentin treated with Prime&Bond NT, Single Bond or Clearfil SE Bond bonding systems. However, Meiers and Kresin [6] found that use of CHX-based cavity disinfectant after tooth preparation, and before the application of a dentin bonding agent might be material specific regarding their interactions with the sealing ability of various dentin-bonding systems. In another study, Gürgan et al. [4], reported that 2% CHX cavity disinfectant application, before or after etching, decreased the shear bond strength of composite resin to dentin but rinsing the cavity disinfectant before adhesive resin application to dentin did not affect the bond strength.

The reason that cavity disinfectants may affect the dentin bond of glass ionomer cements may be explained in the way that these cements attach to dentin and the remnants of CHX could interact with calcium and phosphate present in dentin and therefore inhibit the bonding ability of the GIC. Conventional GICs bond primarily to the inorganic component of tooth structure by a chelation reaction. This involves initial hydrogen bonding followed by the formation of metal ion bridges and is a true physicochemical bond [20]. The results of GIC used in the present study showed µTBS values similar to the other studies in which the bond strength of other high viscosity GICs to dentin was investigated [21,22]. Yet, the results of this study were slightly higher than Tanumiharja et al. [23], and Botelho [24] who found the µTBS of Fuji IX ranging from 5.4 to 9.3 MPa but presenting lower standard deviations. This may be attributed to minor differences in the microtensile bonding technique or the fact that capsulated versions of the GIC material were tested in the study of Tanumiharja et al. [23]. The higher µTBS values of Ketac Molar could be due to the application of Ketac Conditioner for 30 s which removes the smear layer and surface contaminants at the same time as it alters the surface energy and exposes the mineralized tooth structure for the diffusion of the acid and the exchange of ions. The higher standard deviations could be attributed to the hand-mixed GICs, which might have created internal porosities reflecting cohesive failures in the material itself. The ratio of components is controlled by the manufacturer in hand mixed materials as the ratio of powder and liquid varies depending on the differences in cement powder packing densities achieved upon filling the scoop and and the drop of liquid which all might cause large porosities. In the study of Di Hipólito et al. [2], µTBS of the CHX-containing experimental adhesive was found to be significantly higher than the CHX-free adhesive after 24 h aging. The authors concluded that when CHX is incorporated into hydrophilic dental adhesives, it could partially reduce the degradation of the resin-dentin bond 12 months after storage in artificial saliva. In another study, CHX application increased µTBS of Prime & Bond NT and

Single Bond to the acid-etched primary and permanent dentin significantly, while no positive or negative effect was observed for Excite DSC [25]. Considering the non-significant μTBS results of this study using CHX and Prime Bond NT to the both caries-affected or sound dentin, and those of the others, it can be stated that the interaction between the adhesive system and the CHX is also of importance in adhesion to dentin. Certainly, dentin conditioning with an adhesive resin based on 2-step etch and rinse system coupled with the high elasticity modulus of resin-based material PRC compared to GI and RMGI resulted in significantly higher results on both substrates. RMGI showed higher results as opposed to GI yet not significant with and without CHX application. This needs to be verified in higher sample size in future studies. Moreover, pre-test failures were not included in this study but considering such failures as 0 MPa could create statistical distinction between GI and RMGI that needs to be verified.

Laboratory studies that measure dentin bond strengths are principally conducted on ground, flat or non-carious dentin substrates. These substrates are not necessarily representative of the dentin encountered during many restorative procedures in clinical situations. The caries-affected dentin is significantly softer than sound dentin at the same remaining dentin thickness due to the loss of apatite mineral from intertubular dentin [26,27]. It has been reported in previous studies that the adhesion of resin to caries-affected dentin was inferior to that of sound dentin due to weaker collagen and/or weaker resin [28-30]. Therefore, carious dentin substrate was used in order to carry out a more clinically relevant study and the results were compared with those of the sound dentin. Similar trends were observed with other materials in previous studies for the microtensile bond strengths of the tested restorative materials to caries-affected and sound dentin [31,32]. It has to be however noted that caries removal method based on visual screening the slightly pink coloured areas or tactile feeling of the hard surface contact on probing remains to be subjective in all studies dealing with caries-affected dentin substrates. Non-contact measurement methods used for measurement of the hardness level of caries-affected dentin may be correlated with the adhesion results in future studies.

Bond strength results should be also accompanied with failure type analysis. The incidence of predominantly adhesive failure type in the PRC group, indicates that the adhesive resin debonded primarily at the interface between the adhesive and the dentin. On the other hand, in the GI and RMGI groups, cohesive failures in the material was more in common, that could be attributed to the porosity in the materials and the weaker tensile strength of these materials. Possibly the number of defects within the RMGI was less than in high viscosity GI and this explains the higher number of mixed (Score 2) failures followed by cohesive failures in the material (Score 3). One limitation of this study is that failure types were analyzed using stereoscopic microscope images. Detailed analysis of failure types should be performed in the future with more sophisticated microscopy techniques.

Conclusion

From this study, the following could be concluded:

1. The use of 2% chlorhexidine gluconate based cavity disinfectant did not impair the adhesion of the restorative materials tested to sound and caries-affected dentin.
2. Packable resin composite demonstrated significantly higher mean μTBS values than those of glass ionomer and resin-modified glass ionomer but the failure types were more favorable for the latter two.

Clinical Relevance

The application of 2% chlorhexidine gluconate based cavity disinfectant showed no adverse effect on bond strength of glass ionomer, resin-modified glass ionomer and packable resin composite materials on both sound and caries-affected dentin.

Conflict of interest

The authors did not have any commercial interest in any of the materials used in this study.

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Legends

Figures:

Fig. 1. Experimental design of the study consisting the groups and the treatment methods.

Tables:

Table 1. Brands, manufacturers, batch numbers, types and chemical compositions of the materials used in this study

Table 2: μTBS (MPa) values and standard deviations for experimental groups

Table 3. Distribution of failure types (%) in each experimental group. Score 1= adhesive failure between the restorative material and dentin; Score 2= partially adhesive failure between dentin and restorative material and dentin accompanied with cohesive failure in the restorative material (mixed); Score 3= cohesive failure of the restorative material only; Score 4= cohesive failure in dentin

Figures:

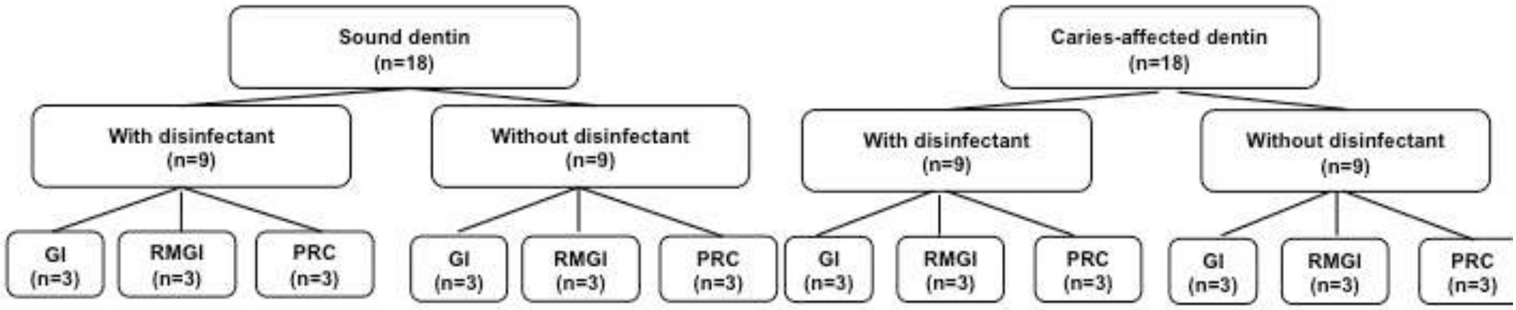

Fig. 1. Experimental design of the study consisting the groups and the treatment methods.

Tables:

Table 1. Brands, manufacturers, batch numbers, types and chemical compositions of the materials used in this study.

Brand (Manufacturer)	Batch number	Type	Chemical Composition
Ketac Molar (3M ESPE, Seefeld, Germany)	01128273	Powder	Calcium aluminium, lanthanum-fluorosilicate glass, acrylic acid-maleic acid copolymer pigments
		Liquid	Water, acrylic acid-maleic acid copolymer, tartaric acid
		Ketac Conditioner	25% polyacrylic acid
Vitremer (3M ESPE, Seefeld, Germany	3303MP-A3	Primer	46% HEMA, 39% ethyl alcohol, 15% Vitrebond copolymer
		Powder	Fluori-aluminosilicate glass, potassium persulfate, ascorbic acid
		Liquid	50% polyacrylic acid copolymer, 20% HEMA, water, 13% carboxylic acid copolymer
Surefil (Dentsply/Caulk Milford, DE, USA)	010320	Packable resin composite	bis-GMA, TEGDMA, bis-EMA, barium floroaluminoborosilicate glass fumed silica, stabilizers, photoinitiators
Prime & Bond NT (Dentsply De Trey, Konstanz, Germany)	030822	Conditioner	36% phosphoric acid gel
		Bond	PENTA, UDMA, Resin R5-62-1, T-resin, D-resin, nanofiller, initiators, stabilizer, cetylaminehydrofluoride, acetone
Consepsis (Ultradent, South Jordan, UT, USA)	80100	Chlorhexidine gluconate antibacterial solution	2% chlorhexidine gluconate

Table 2. μTBS (MPa) values and standard deviations for experimental groups.

Groups and materials	Mean μTBS (SD) **Sound dentin**	Mean μTBS (SD) **Caries-affected dentin**
High viscosity GIC (KetacMolar)		
With disinfectant	11.3 (6.72)[a]	10.2 (6.52)[a]
Without disinfectant	12.2 (6.25)[a]	11.1 (7.23)[a]
Resin modified GIC (Vitremer)		
With disinfectant	16.2 (6.38)[a]	15.6 (7.85)[a]
Without disinfectant	19.8 (6.46)[a]	18.3 (6.39)[a]
Packable composite resin (Surefil)		
With disinfectant	38.2 (7.24)[b]	36.9 (6.74)[b]
Without disinfectant	39.6 (5.28)[b]	38.7 (5.43)[b]

Different small letters present significantly different groups ($p<0.05$).

Table 3. Distribution of failure types (%) in each experimental group. Score 1= adhesive failure between the restorative material and dentin; Score 2= partially adhesive failure between dentin and restorative material and dentin accompanied with cohesive failure in the restorative material (mixed); Score 3= cohesive failure of the restorative material only; Score 4= cohesive failure in dentin.

Experimental Groups			**Failure Types***			
			Score 1 (%)	Score 2 (%)	Score 3 (%)	Score 4 (%)
Glass ionomer (Ketac Molar)	Sound dentin	Without CHX	-	14	86	-
		With CHX	4	16	80	-
	Caries-affected dentin	Without CHX	-	6	94	-
		With CHX	3	13	84	-
Resin-modified glass ionomer (Vitremer)	Sound dentin	Without CHX	12	26	62	-
		With CHX	23	23	54	-
	Caries-affected dentin	Without CHX	8	25	67	-
		With CHX	12	25	63	-
Packable resin composite (Surefil)	Sound dentin	Without CHX	89	11	-	-
		With CHX	78	22	-	-
	Caries-affected dentin	Without CHX	98	2	-	-
		With CHX	93	7	-	-